IN THE UNITED STATES PATENT AND TRADEMARK OFFICE		
	Application Number	09/453,801
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	First Named Inventor	Saswati CHATTERJEE, et al.
	Group Art Unit	1636
	Examiner Name	G. Leffers, Jr.
	Attorney Docket Number	1954-287

Title of the Invention: *A METHOD OF GENETICALLY MODIFYING VERY PRIMITIVE QUIESCENT HUMAN HEMATOPOIETIC STEM CELLS*

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AMENDMENT

Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

In response to the non-final Office Action of July 18, 2001, Applicants respectfully request that the Examiner reconsider the application in view of the following amendments and remarks. Declarations under 37 C.F.R. §§ 1.131 and 1.132 accompany this response.

In the Claims:

Please cancel claims 24-33 and amend claims 12, 13, and 23 as follows:

12. (Amended) A method according to claim 11, wherein the transferred DNA remains integrated into the genome of the multi-potential hematopoietic stem cells for at least 4 weeks.

13. (Amended) A method according to claim 11, wherein the transferred gene remains integrated into the genome of the multi-potential hematopoietic stem cells for at least 8 weeks.

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23. (Amended) A method for stably transferring DNA into multi-potential hematopoietic stem cells in the G0 phase of the cell cycle, which comprises transducing said multi-potential hematopoietic stem cells with an adeno-associated virus vector that contains said DNA, wherein said multi-potential hematopoietic stem cells are $CD34^{+++}CD38^{-}$ cells in the G0 phase of the cell cycle.

REMARKS

Claims 1-33 are pending in this application, however claims 24-33 are withdrawn from consideration as drawn to non-elected subject matter. Claims 24-33 are canceled herein. Applicants reserve the right to continue prosecution of the canceled subject matter in a divisional application.

Claims 1-6, 11-14 and 22-23 are rejected under 35 U.S.C. § 102(a) as anticipated by Wong et al. This reference, authored by the inventors of this application, bears a date of November 15, 1998, only one month prior to the effective filing date of this application, although it may not have been available publicly until December 4, 1998. Enclosed herewith is a declaration pursuant to 37 C.F.R. § 1.131, signed by the inventors of the present application. In this declaration, the inventors state that the claimed invention was made prior to November 15, 1998, and submit in support of this statement copies of pages from the laboratory notebooks of Christie Ann Wong with electronic data files and photographs showing production of the cells, the nature of the cells and the stable transduction of the cells transduced. The entries of these laboratory notebook pages were made at the time the experimental work was carried out and were dated contemporaneously. All the dates on the original pages pre-date November 15, 1998. The dates on the photocopies of the pages have been redacted,

but the pages are otherwise accurate copies of the original pages. These pages, along with the accompanying data, show production of $CD34^{+++}CD38^{-}$ hematopoietic true stem cells residing in the G0 phase of the cell cycle and assays demonstrating their status; transduction of these cells using rAAV; and stable integration of the rAAV sequences into these cells. All steps necessary to render the subject invention complete were made in the United States prior to or by the date on which the last of the original pages of Exhibit 1 were dated.

In view of this declaration under 37 C.F.R. § 1.131, Applicants respectfully submit that the disclosures of the Wong et al. reference have been antedated and that the Wong et al. reference cannot be considered as prior art against the pending claims. Applicants respectfully submit that the rejection of claims 1-6, 11-14 and 22-23 as anticipated by Wong et al. is no longer proper. Applicants therefore request that this rejection be withdrawn.

Claims 1-23 are rejected under 35 U.S.C. § 102(b) as being anticipated by Fisher-Adams et al. The Office Action states that this reference teaches the construction and use of AAV vectors for transduction of human bone marrow or umbilical cord $CD34^{+}$ hematopoietic progenitor cells obtained by selection with anti-$CD34^{+}$ antibody. The cells are described in the Office Action as 70-95% pure. The Office Action further states that the method of *initially* selecting the $CD34^{+}$ cell population described by Fisher-Adams et al. is essentially the same means applied in the present application and that the media for culture and transduction also are the same. From this, the Office Action concludes that the skilled person would necessarily expect a sub-population of quiescent $CD34^{+}$ cells residing in G0 and a sub-population of $CD34^{+++}CD38^{-}$ cells would be present in the cells used for the transduction, and that these cell sub-populations

would remain quiescent during the transduction. No specific disclosure in the reference is cited to indicate that $CD34^{+++}CD38^{-}$ cells residing in G0 are in fact transduced.

For a rejection under § 102(b) to be proper, the reference cited as anticipating must teach or disclose each and every claim limitation. M.P.E.P. § 2131. The Office has not alleged that Fisher-Adams et al. teach transduction of multi-potential hematopoietic stem cells in the G0 phase of the cell cycle, cells of the type which Applicants claim. Applicants respectfully submit that a *prima facie* case of anticipation under 35 U.S.C. § 102(b) is not made out when the Office has pointed to nothing in the reference which teaches one or more claim element. The rejection thus should be withdrawn on this basis alone.

The Office has indicated that Applicants must show a novel or unobvious difference between the claimed product and the products of the prior art. The Office has presented no information that indicates that $CD34^{+++}CD38^{-}$ cells residing in G0 have been transduced and therefore has not met its burden in showing the claims have been anticipated under 35 U.S.C. § 102. Nevertheless, Applicants herein present evidence of a novel and unobvious difference between the claimed product and the products of the prior art, as requested by the Office, including a Declaration under 37 C.F.R. § 1.132 ("Chatterjee Decl.").

Inventors of the present invention, Kamehameha K. Wong, Jr. and Saswati Chatterjee, are authors of Fisher-Adams et al. Chatterjee Decl. ¶ 4. The reference describes transduction of cells semi-purified using anti-$CD34^{+}$ antibodies. Chatterjee Decl. ¶ 4. These cells are not a population of true stem cells as were the cells transduced in the present invention. Chatterjee Decl. ¶ 4. This fact was recognized at the time this paper was published. Chatterjee Decl. ¶ 4. In addition, the method used

to isolate the $CD34^+$ cells was not the same method used and described in the present application. Chatterjee Decl. ¶ 4.

The factual assertions contained in the Office Action regarding the significance of Fisher-Adams et al. are not correct. Chatterjee Decl. ¶ 5. The conclusion that a person skilled in the art would expect that quiescent, true stem cells residing in G0 would be transduced by the methods of Fisher-Adams et al. is unjustified. Chatterjee Decl. ¶ 5. A person knowledgeable about methods for transducing and attempts to transduce true hematopoietic stem cells at the time of Fisher-Adams et al. and before the filing of the present application would not expect that these cells would have been stably transduced. Chatterjee Decl. ¶ 5.

The methods of Fisher-Adams et al. involved incubation with a mouse anti-CD34 antibody, followed by panning on sheep anti-mouse Ig-coated flasks. Chatterjee Decl. ¶ 6. To obtain the cells used in the present invention, cells were selected using Miltneyi columns followed by appropriate live staining and flow sorting of $CD34^{+++}CD38^-/CD34^{+++}$ G0 cells. Chatterjee Decl. ¶ 6. These cells were sorted based on DNA and RNA content to segregate out only those $CD34^+$ cells which were in the G0 phase (see specification, p 17). Chatterjee Decl. ¶ 6. These G0 cells then were further examined for CD34 and CD38 status and sorted to remove $CD38^+$ staining cells. Chatterjee Decl. ¶ 6. This three-step procedure results in a highly purified population of G0 cells from which G1 cells have been removed. Chatterjee Decl. ¶ 6. These cells are different, therefore, from any cells which have been transduced in the prior art or in Fisher-Adams et al. Chatterjee Decl. ¶ 6.

The G0 cell cycle status of these purified $CD34^{+++}CD38^-$ cells was confirmed a second time based on DNA and RNA content (see specification, p 18, line 23; p 19,

lines 1-3 and Tables 1 and 4). Chatterjee Decl. ¶ 7. The quiescent, non-cycling nature of the cells used also was confirmed using lipophilic membrane dyes, further demonstrating their G0 status. Chatterjee Decl. ¶ 7. Applicants' methods resulted in isolation of 0.06% of the total marrow cells, or 6 in 10,000 cells. Chatterjee Decl. ¶ 7. The frequency of true stem cells is estimated to be between 1 in 10,000 and 1 in 100,000 cells. Chatterjee Decl. ¶ 7. Thus, Applicants have approached the limit of purification. Chatterjee Decl. ¶ 7. The evidence, clearly spelled out in the specification of the present application as filed, is more than sufficient to differentiate the cells used by Applicants with the cells of Fisher-Adams et al. Chatterjee Decl. ¶ 7.

The cells transduced by Fisher-Adams et al. were semi-purified $CD34^+$ cells of undetermined cell cycle status. Chatterjee Decl. ¶ 8. There is nothing in Fisher-Adams et al. to indicate that quiescent, non-cycling pluripotent true stem cells were transduced by the methods used, nor did the authors of Fisher-Adams et al. claim pluripotent stem cell transduction. Chatterjee Decl. ¶ 8. The present specification, page 10, lines 5-14, discusses this in reference to the disclosures of Fisher-Adams et al. Chatterjee Decl. ¶ 8. In contrast, the cells transduced and the methods claimed in the present application, require G0 cell cycle status. Chatterjee Decl. ¶ 8.

In addition, the CD34+ cells of Fisher-Adams et al. were cultured in media with cytokines (IL-3, 10ng/ml; IL-6, 5 ng/ml and GM-CSF, 1ng/ml) which are recognized not to support stem cells, since no Stem Cell Factor was present. Chatterjee Decl. ¶ 9. Thus, hematopoietic stem cells could not be transduced under these conditions. Chatterjee Decl. ¶ 9. In contrast, Applicants methods included 1ng/ml Stem Cell Factor (SCF; see Example 3). Chatterjee Decl. ¶ 9. Under these conditions stem cell survival is expected and was observed. Chatterjee Decl. ¶ 9. Thus, Applicants' culture

conditions do allow transduction of stem cells while those used by Fisher-Adams et al. do not. Chatterjee Decl. ¶ 9.

Prior to Applicants' invention claimed here, transduction of extremely primitive, G0, quiescent, pluripotent stem cells had not been demonstrated. Chatterjee Decl. ¶ 10. Applicants' invention therefore is novel and nonobvious. Chatterjee Decl. ¶ 10. Nothing cited by the Office in Fisher-Adams et al. teaches anything to the contrary. Chatterjee Decl. ¶ 10. Applicants therefore submit that the cells claimed in the present application possess a novel and nonobvious difference from those of the Fisher-Adams et al. reference and request that the Office withdraw the rejection over this reference.

Claims 1-6, 11-15 and 22-23 are rejected under 35 U.S.C. § 102(b) as anticipated by Zhou et al. The Office states that Zhou et al. teach methods of gene transfer into subsets of hematopoietic progenitor cells using AAV-2 vectors, the cells used for gene transfer being $CD34^+$ cells selected by binding to a CD34-specific antibody, *similarly to* those used in Fisher-Adams et al. Therefore, the arguments discussed above apply equally here. The Office has not made out a *prima facie* case of anticipation but improperly places the burden on Applicants to submit evidence of lack of anticipation. Applicants respectfully submit that this rejection is not proper, but submit the attached Declaration under 37 C.F.R. § 1.132 ("Chatterjee Decl.") to provide evidence of a novel and unobvious difference between the claimed product and the products of the prior art as requested.

The Zhou et al. reference is cited by the Office as teaching methods of gene transfer into subsets of hematopoietic progenitor cells using AAV-2 vectors, the cells used for gene transfer being $CD34^+$ cells selected by binding to a CD34- specific antibody, *similarly to* those used in Fisher-Adams et al. Zhou et al. identified DNA

fragments in various clones obtained from their $CD34^+$ cells after transduction. Chatterjee Decl. ¶ 11. These progeny clones necessarily had been through one or more cell cycle, therefore the cells could not have been non-cycling. Chatterjee Decl. ¶ 11. The authors were only able to conclude that their results "*suggest* that relatively slow *or* noncycling hematopoietic progenitor cell populations in cord blood are susceptible to infection" by AAV, p 1872, col. 1, lines 33-36 (emphasis added). Chatterjee Decl. ¶ 11. The authors do not claim that they transduced true stem cells. Chatterjee Decl. ¶ 11. In fact, it is extremely unlikely that the cells transduced by Zhou et al. could have been quiescent cells residing in G0, given the transfection conditions. Chatterjee Decl. ¶ 11. Zhou et al. used what the Office characterizes, on page 5, line 21, as "low levels" of cytokines (100 U/ml Epo, 100 U/ml IL-3, 100 U/ml GM-CSF). Chatterjee Decl. ¶ 11. The levels, however, are 10-fold higher than those used by Applicants. Chatterjee Decl. ¶ 11. As explained in the specification, page 14, lines 9-23, these higher cytokine levels result in mitosis (loss of G0 cell cycle status). Chatterjee Decl. ¶ 11. Contrary to the conclusion stated in the Office Action, one of skill in the art would *not* expect that the population of $CD34^+$ cells described as transduced by Zhou et al. are quiescent and in G0 cell cycle status. Chatterjee Decl. ¶ 11.

Furthermore, Zhou et al. transduced CFU-C in a short-term (14 day) methyl cellulose colony forming assay. Chatterjee Decl. ¶ 12. It is well recognized that these cells do not represent either primitive or quiescent cells. Chatterjee Decl. ¶ 12. These cells have been shown unequivocally to represent lineage committed cells well into the differentiation process. Chatterjee Decl. ¶ 12. $CD34^{+++}CD38^-$ cells residing in G0 are very primitive and do not readily give rise to colonies in a 14 day CFU-C assay. Chatterjee Decl. ¶ 12. Thus, a skilled artisan would have concluded that Zhou et al.

demonstrated rAAV transduction of cycling, lineage committed, differentiating cells and nothing more. Chatterjee Decl. ¶ 12. Applicants, on the other hand, have shown transduction of extremely primitive hematopoietic cells in the G0 phase. Chatterj e Decl. ¶ 12. The cells claimed by Applicants therefore have a novel and unobvious difference from those in the Zhou et al. reference. Chatterjee Decl. ¶ 12.

Because Zhou et al. do not teach the transduction of cells in the G0 phase, this reference does not anticipate any of the claims of the present application under 35 U.S.C. § 102(b). See M.P.E.P. § 2131. Applicants request that the rejection of claims 1-6, 11-15 and 22-23 as anticipated by Zhou et al. be withdrawn.

Claims 1-4, 6-7, 11-15 and 22-23 are rejected under 35 U.S.C. § 102(b) as anticipated by Luhovy et al. The Office states that Luhovy et al. teach the stable introduction of genes into hematopoietic stem cells via transduction with an AAV vector. The Office reasons, equivalent to the reasoning given in the rejections over Fisher-Adams et al. and Zhou et al., that the *initial* selection of $CD34^+$ cells described by Luhovy et al., is essentially the same as that used by Applicants, and that Luhovy et al. isolate cells lacking Lin and Thy differentiation markers. The Office does not, however, allege that the cells transduced are in the G0 phase. Thus, all the remarks which pertain to Fisher-Adams et al. and Zhou et al. apply to Luhovy et al. Applicants respectfully submit that this rejection is not proper because the Office has not made out a *prima facie* case of anticipation by citing teachings in the prior art reference which disclose all claim limitations as required by M.P.E.P. § 2131. As requested by the Office, however, Applicants submit the attached Declaration under 37 C.F.R. § 1.132 ("Chatterjee Decl.") to provide evidence of a novel and nonobvious difference between the claimed product and the products of the prior art.

Luhovy et al. used methods very similar to those of Zhou et al. Chatterjee Decl. ¶ 13. They transferred LacZ sequences into $CD34^+$ Lin^-Thy^- cells from presumably adult bone marrow donors. Chatterjee Decl. ¶ 13. None of the methods used by Luhovy et al. were designed to ensure the G0 cell cycle status of the cells: the antibody-purified $CD34^+$ cells were treated only to remove certain specific fully differentiated cell types (see p 25, col. 1). Chatterjee Decl. ¶ 13. These cells therefore were not the G0-residing cells used by Applicants as described above and in the specification as filed. Chatterjee Decl. ¶ 13.

The LTC-IC assay employed by Luhovy et al. suggests that the population of cells they used contained more primitive cells than those used by Zhou et al., however the bulk of evidence indicates that cells from LTC-IC assays are still lineage committed and do not represent true stem cells. Chatterjee Decl. ¶ 14. The fact that retrovirus vectors readily transduce this population indicates that these cells are, in fact, not stem cells residing in G0. Decl. ¶ 14. Retrovirus vectors do not transduce true stem cells. Therefore, the conclusion that the Luhovy et al. methods transduced cells in G0 cell cycle status, Office Action, page 8, lines 12-19, is completely unwarranted. Chatterjee Decl. ¶ 14.

The cells claimed in the present application therefore have a novel and unobvious difference from those of the Luhovy et al. reference. Chatterjee Decl. ¶ 15. Thus, as for the other references cited, Luhovy et al. do not teach the transduction of G0 cells and can not anticipate the claims of the present application. Chatterjee Decl. ¶ 15. Applicants therefore request that the rejection be withdrawn.

In summary, none of the references cited by the Office as anticipating teach transduction of G0 cells. Chatterjee Decl. ¶ 16. The methods used by these authors

are not the same as those used by Applicants and lack several steps used by Applicants purify hematopoietic stem cells that exist in G0. Chatterjee Decl. ¶ 16. Transduction of cells in G0 was not shown. Chatterjee Decl. ¶ 16. Expansion of the cells used in the prior art revealed that some cell populations had been transduced and carried the foreign DNA, however these colonies of transduced cells are recognized to result from cycling, at least partially differentiated cells. Chatterjee Decl. ¶ 16. A careful comparison of the methods described by Fisher-Adams et al., Zhou et al. and Luhovy et al. clearly shows that the transduced cell populations are not equivalent to the cells claimed in the present application and that no prior art reference teaches methods for transducing cells in the G0 phase as Applicants have claimed. Chatterjee Decl. ¶ 16.

As further proof that the cells used in this application are true stem cells, Applicants have demonstrated the cell cycle status and quiescent nature of their cells by two different assays: immunophenotyping and in vivo stem cell assays. Chatterjee Decl. ¶ 17. These assays show that the cells described and claimed in the present application are able to engraft immune-deficient mice in secondary transplants. Chatterjee Decl. ¶ 17. This is the only available assay to show that cells are true stem cells. Chatterjee Decl. ¶ 17. Results are attached as Exhibit 2 to the accompanying Chatterjee declaration.

The assay was performed as follows. Chatterjee Decl. ¶ 18. Purified $CD34^{+++}CD38^{-}$ human cord blood cells in the G0 phase, transduced according to the methods of this invention, were transplanted into sublethally irradiated NOD/SCID mice. Chatterjee Decl. ¶ 18. Engraftment with the transduced human cells, multilineage differentiation of the cells and the ability of the cells engrafted in the bone marrow to give rise to CFU-C

colonies were examined. Chatterjee Decl. ¶ 18. The presence of rAAV sequences in SCID repopulating cells and marrow CFU-C also were analyzed. Chatterjee Decl. ¶ 18.

The results clearly showed engraftment of these highly primitive $CD34^{+++}CD38^{-}$ cells as determined by the frequency of human $CD45^{+}$ cells in the mouse bone marrow and spleen. See Figures 1 and 2. Chatterjee Decl. ¶ 19. Engraftment levels rose with time after transplantation, revealing the primitive nature of the transplanted cells. Chatterjee Decl. ¶ 19. The data show that these cells can engraft and differentiate in the marrow of NOD/SCID mice for at least 25 weeks after bone marrow transplant. Chatterjee Decl. ¶ 19. More importantly, the engrafted cells differentiated into different hematopoietic lineages. See Figure 3. Chatterjee Decl. ¶ 19. $CD14^{+}$ and $CD33^{+}$ myeloid and $CD19^{+}$ B lymphocytes were observed consistently. Chatterjee Decl. ¶ 19. $CD34^{+}$ progenitor cells also were observed in vivo even at 25 weeks after transplantation. See Figure 3. Chatterjee Decl. ¶ 19. The presence of rAAV sequences in these different repopulating lineages were determined by amplification of the vector sequences, proving that the transplanted and stably transduced cells were true stem cells, capable of multipotential differentiation. See Table 1. Chatterjee Decl. ¶ 19.

The self-renewal capacity of the rAAV transduced cells and their progeny was determined by plating the cells in colony forming assays. Chatterjee Decl. ¶ 20. CFU-GEMM (colony forming unit -- granulocyte, erythrocyte, monocyte, macrophage) colonies were obtained from the transduced cells, again showing that the transduced cells were highly primitive, multipotential cells. See Figure 4. Chatterjee Decl. ¶ 20.

Bone marrow cells were harvested from the primary hosts approximately 7-8 weeks after bone marrow transplant and infused into secondary sublethally irradiated

recipients. Chatterjee Decl. ¶ 21. Analysis of these secondary recipients revealed the presence of rAAV sequences in the myeloid and lymphoid cells in the bone marrow and spleen of 4 out of 4 animals, indicating that self-renewing *true stem cells* capable of extended survival had been transduced in the original inoculum. See Figure 5. Chatterjee Decl. ¶ 21. Importantly, rAAV sequences were detected in the $CD34^+$ population in the bone marrow of a secondary recipient, indicating that the methods claimed in the present application are able to and did transduce *true stem cells* using rAAV. Chatterjee Decl. ¶ 21.

These new data, provided in the attached Declaration under 37 C.F.R. § 1.132, conclusively demonstrate the stable transduction of the highly primitive, quiescent stem cells residing in G0. Chatterjee Decl. ¶ 22. Applicants respectfully submit that the discussion and additional data provided here and in the attached declaration under 37 C.F.R. § 1.132 are more than sufficient to show the novel and nonobvious differences between the product claimed in this application and those of the cited prior art references. Chatterjee Decl. ¶ 22. Applicants therefore request that the rejections over Fisher-Adams et al., Zhou et al. and Luhovy et al. be withdrawn.

Claims 1-23 are rejected under 35 U.S.C. § 112, second paragraph, as indefinite. Claims 1 and 23 are considered indefinite for the use of the term "stably transfecting," which the Office considers undefined in the specification. On page 14 of the specification, at lines 27-28, the stability of the integration of transferred DNA is described as "at least 4 weeks or 8 weeks, or much longer." Applicants therefore submit that the term "stably transferring" DNA does not render the claims indefinite. Further, claim 12, which is indirectly dependent on claim 1, specifically claims integration "for at least 4 weeks." Claim 1 therefore must be read at least as broadly as

claim 12. Applicants respectfully submit that amendment is not necessary since the specification does define the term. Claims 1 and 23 fully comply with 35 U.S.C. § 112, second paragraph. Applicants therefore request that the rejection of the claims on this basis be withdrawn.

Claim 2 is rejected as vague and indefinite. The Office specifically points to the term "substantial" with respect to levels of differentiation or mitotic activity as indefinite. The term "substantially" has been recognized by the Office as definite. See M.P.E.P. § 2173.05(b). This is particularly true when the specification contains general guidelines as to what the term means. See In re Nehrenberg, 126 U.S.P.Q. 383 (C.C.P.A. 1960); In re Mattison, 184 U.S.P.Q. 484 (C.C.P.A. 1975), M.P.E.P. § 2173.05(b). The specification, page 15 at lines 9-19, provides more than ample guidance for the person of ordinary skill to understand what is meant by this term, describing the differentiation and mitosis both in terms of time and percentage. Applicants submit that this term is not indefinite and that claim 2 complies fully with the requirements of 35 U.S.C. § 112, second paragraph. Applicants therefore respectfully request that the rejection on this basis be withdrawn.

Claims 3-5 are rejected as indefinite for use of the term "about" as applied to the duration of the transduction period. This term is recognized as definite by both the Office and the case law. See M.P.E.P. § 2173.05(b); Ex parte Eastwood, 163 U.S.P.Q. 316 (Bd. App. 1968); W. L. Gore & Assoc., Inc., 220 U.S.P.Q. 303 (Fed. Cir. 1983). This is particularly so where the specification provides guidance regarding the range that is covered by the term. The specification, page 13, line 29 to page 14, line 8 describes the transduction times which are useful and which are preferred. The specification relates these times to the quiescent and mitotically dormant state which

the cells should maintain during transduction, and therefore provides more than adequate indication of the transduction times which may be used. This instruction is repeated at page 24, lines 6-16, providing additional guidance as to the functional time limits which define useful transduction times. Applicants respectfully submit that claims 3-5 fully comply with 35 U.S.C. § 112, second paragraph, and request that the rejection on this basis be withdrawn.

Claims 7-10 are rejected under 35 U.S.C. § 112, second paragraph, as indefinite with respect to use of the term "about" to describe cytokine levels. Applicants refer the Office to the case law and M.P.E.P. citations given above. The specification, page 14, lines 9-23, discuss appropriate cytokine levels which are useful with the inventive methods. The description guides the reader to use levels of cytokine which are not so high as to stimulate mitosis, but not so low as to cause cell death, and provides ranges which can be used. A person of skill would use these guidelines to interpret the claims and would easily understand what levels of cytokine are appropriate and claimed. Considering the knowledge of one of ordinary skill and the guidance in the specification, Applicants submit that this term is not indefinite under 35 U.S.C. § 112, second paragraph. Applicants therefore submit that the rejection on this basis should be withdrawn.

Claim 6 has been rejected as indefinite under 35 U.S.C. § 112, second paragraph, for use of the term "low cytokine levels," which has not been clearly defined. The disclosures discussed above define specifically what is meant by "low cytokine levels." Applicants also call the Office's attention to page 13, line 29 to page 14, line 1, which defines low cytokine levels as levels at which the hematopoietic stem cells in culture remain quiescent and mitotically dormant for up to 48 hours or longer. Page 14, lines

9-23 describe the cytokine level conditions for the inventive method and provide guiding ranges for the cytokines. Further, a preferred example of "low cytokine concentrations" which fall within the disclosed ranges are provided at page 29, lines 5-6. Applicants submit that these disclosures provide ample guidance to instruct the skilled person what is meant by this term. Applicants therefore request that the rejection of claim 6 under 35 U.S.C. § 112, second paragraph, be withdrawn.

Claims 12-13 have been rejected as vague and indefinite for use of the term "capable of." Applicants have amended claims 12 and 13 to avoid use of this term and believe that this amendment obviates the rejection. Applicants therefore request the rejection of claims 12 and 13 on this basis be withdrawn.

Claim 14 is rejected as indefinite for use of the term "$CD34^{+++}$." The claim recites $CD34^{+++}CD38^{-}$ cells, rather than "$CD34^{+++}$" cells. The term "$CD34^{+++}CD38^{-}$" is fully defined and described in the specification. See specification, page 13, lines 11-13, Figure 3 and page 17, lines 33-36. Applicants submit that this disclosure more than adequately defines the term as used in claim 14 and provides detailed instructions on how to obtain the cells. Applicants therefore respectfully submit that claim 14 fully complies with 35 U. S. C. § 112, second paragraph, and request that the rejection on this basis be withdrawn.

Claim 16 is rejected as indefinite for use of the term "derived from." The Office has requested clarification of the nature and number of steps required to generate an AAV vector "derivative" of the vector CWRSV. Applicants submit that it is well within the skill of the ordinary artisan in the field of molecular biology to design and construct any number of AAV vectors from CWRSV, even with no guidance whatsoever. In this case, however, the disclosures of the specification provide several examples of such vectors

and source material for further background information if necessary, as well as description of CWRSV. See, for example, page 9, lines 19-21; page 15, line 35 to page 16, line 3; page 21, line 28 to page 22, line 7; page 35, line 31 to page 36, line 10; and Figure 7. Applicants therefore submit that use of this term does not render the claim indefinite since the meaning would be clearly and immediately understood by one of skill in the art. The specification fully describes, in text, by reference, and through use of examples, vectors derived from and based on CWRSV, making the term that much more clear to the readers. Applicants submit that claim 16 fully complies with the requirements of 35 U.S.C. § 112, second paragraph and request that the rejection on this basis be withdrawn.

Claim 23 is rejected as indefinite due to a word processing error which resulted in duplication of the phrase "which comprises transducing said multi-potential hematopoietic stem cells." Applicants have corrected this error by amendment and request the rejection of this claim be withdrawn.

Applicants submit that the indefiniteness rejections of claims 2-10, 14 and 16 should be withdrawn as improperly made, since the rejections were based on lack of definition or guidance in the specification and the general knowledge of the skilled person, and the location of such definition and guidance has been pointed out. Applicants are not required to provide the precise definition of terms in the claims when the meaning of these terms are clear to one of ordinary skill, either from general knowledge in the art or through information provided by Applicants in the specification and/or drawings of the application. Definiteness of claim language must be analyzed in light of the application disclosure, the prior art and the interpretation that would be given the language by the person of ordinary skill. M.P.E.P. § 2173.01. Under these criteria, Applicants

respectfully submit that these claims define the invention sought to be claimed in terms clear to the ordinary practitioner in the art. Applicants therefore request that the rejection of these claims under 35 U.S.C. § 112, second paragraph, be withdrawn.

Applicants believe that the claims now are in condition for allowance and respectfully request favorable consideration.

Respectfully submitted,

By: _____

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Enclosure: Mark-up of Claims
Declaration Under 37 C.F.R. § 1.131
Declaration under 37 C.F.R. § 1.132

1954-287.am1

Mark-up of Claims:

12. (Amended) A method according to claim 11, wherein the transferred DNA [is capable of remaining] remains integrated into the genome of the multi-potential hematopoietic stem cells for at least 4 weeks.

13. (Amended) A method according to claim 11, wherein the transferred gene [is capable of remaining] remains integrated into the genome of the multi-potential hematopoietic stem cells for at least 8 weeks.

23. (Amended) A method for stably transferring DNA into multi-potential hematopoietic stem cells in the G0 phase of the cell cycle, which comprises transducing said multi-potential hematopoietic stem cells[, which comprises transducing said multi-potential hematopoietic stem cells] with an adeno-associated virus vector that contains said DNA, wherein said multi-potential hematopoietic stem cells are $CD34^{+++}CD38^{-}$ cells in the G0 phase of the cell cycle.